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WRAMPing Up Calcium in Migrating Cells by Localized ER Transport

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Morphological plasticity and front-rear polarity are essential for directed cell migration. In this issue of *Developmental Cell*, Witze et al. (2013) demonstrate that Wnt5a-mediated signaling induces localization of the cortical endoplasmic reticulum to the trailing edge of melanoma cells and mediates calcium flux, rear detachment, and motility.

Polarized cell migration is necessary for embryonic morphogenesis, leukocyte trafficking, and tumor cell invasion. It is well established that polarization of motile cells is defined by the formation of a leading-edge protrusion and detachment of the trailing edge. However, there remain gaps in our understanding of how cues from the extracellular environment are propagated into polarized regions of front-rear signaling necessary for directed cell migration.

One of the key regulators of polarized signaling involves gradients of calcium (Ca^{2+}) ion flux. Transient flickers of Ca^{2+} at the leading edge are driven by guidance cues such as chemokines. This Ca^{2+} flux has been implicated in leading-edge directional decision making by motile cells (Wei et al., 2012). Substantial evidence also supports the idea that an elevated gradient of Ca^{2+} occurs at the rear of some cell types and is important for rear detachment (Lee et al., 1999). The mechanisms that regulate Ca^{2+} flux at the cell's trailing edge are currently unknown. Work published in this issue of *Developmental Cell* from Witze et al. (2013) demonstrates that the extracellular ligand, Wnt5a, mediates the rearward localization of the endoplasmic reticulum (ER) that is necessary for directed Ca^{2+} release at the trailing edge of motile melanoma cells.

Some extracellular cues, including chemokines, provide leading-edge guidance and promote cell protrusion during directed migration. However, in this case, Witze and colleagues (2013) show that Wnt5a coordinates cell polarity, at least in part, by affecting the posterior of the cell. Wnt5a, a noncanonical member of the Wnt family, has been implicated in melanoma metastasis and has been shown to drive cell migration by regulating the actin cytoskeleton. Witze and colleagues (2013) identified a Wnt-receptor-actin-myosin-polarity (WRAMP) structure that localizes to the rear of melanoma cells following activation by Wnt5a. In the current study, through proteomic approaches, the investigators found that the WRAMP structure contains proteins involved in actin dynamics and cell adhesion (IQGAP1, filamin-A, myosin II, talin-1), microtubule dynamics (nuclear migration protein nudC), membrane trafficking (clathrin heavy chain like-1), enzymatic function (calpain-2, PAK2, Erk2), and ER trafficking (COP-Iβ). Identifying COP-Iβ as part of the WRAMP proteome was surprising and led Witze and colleagues (2013) to speculate that Wnt5a induces the polarization of the ER to the cell's rear. Indeed, live imaging revealed that Wnt5a-induced ER mobilization to the trailing edge precedes Ca^{2+} gradients and subsequent membrane retraction.

The finding that the ER polarizes to the trailing edge of cells is intriguing. The ER/Golgi complex is generally considered to be important at the front of the cell as the main source of new protein production. It also supplies the leading edge with receptors and other components to optimize membrane protrusion. However, the ER is highly motile and dynamic within cells and has diverse roles, including regulating Ca^{2+} homeostasis and focal adhesion dynamics (Bola and Allan, 2009). Indeed, the temporal and spatial functions of ER tubules are not well defined. Notably, Witze et al. (2013) demonstrate that ER tubules localize to the rear of the cell, where they control Ca^{2+} signaling to mediate substrate detachment.

Focal adhesion turnover and actomyosin contraction at the trailing edge of polarized cells are both Ca^{2+}-regulated processes that mediate cell migration. Interestingly, Wnt5a induces an almost 10-fold increase in localized Ca^{2+} concentrations within the WRAMP structure at the cell's rear. The proteomics analysis revealed the localization of key Ca^{2+} targets such as calpain within the WRAMP structure. Calpain has been shown to be critical for substrate detachment at the rear of mesenchymal cells through the proteolysis of specific substrates like talin (Franco et al., 2004). Thus, it seems that the WRAMP structure provides a localized environment for temporally and spatially restricting calpain's enzymatic activity in close proximity to its substrates. Consistent with this, Witze et al. (2013) found that calpain-2 was necessary for membrane retraction at the rear of the cell, downstream of Wnt5a.

It is not clear whether the rearward localization of the ER is conserved in other types of motile cells such as amoeboid cells like lymphocytes and neutrophils. Amoeboid cells migrate under conditions of low adhesion and form a distinctive rearward protrusion known as the uropod. It is known that in some amoeboid cells, like lymphocytes, mitochondria polarize to the rear and may help to drive actomyosin contractility in the uropod (Campello et al., 2006). It is possible that the posterior ER/Ca^{2+} signaling is also involved in the leukocyte uropod. Interestingly, calpain proteases are not involved in rear detachment and motility of neutrophils (Lokuta et al., 2003), suggesting that

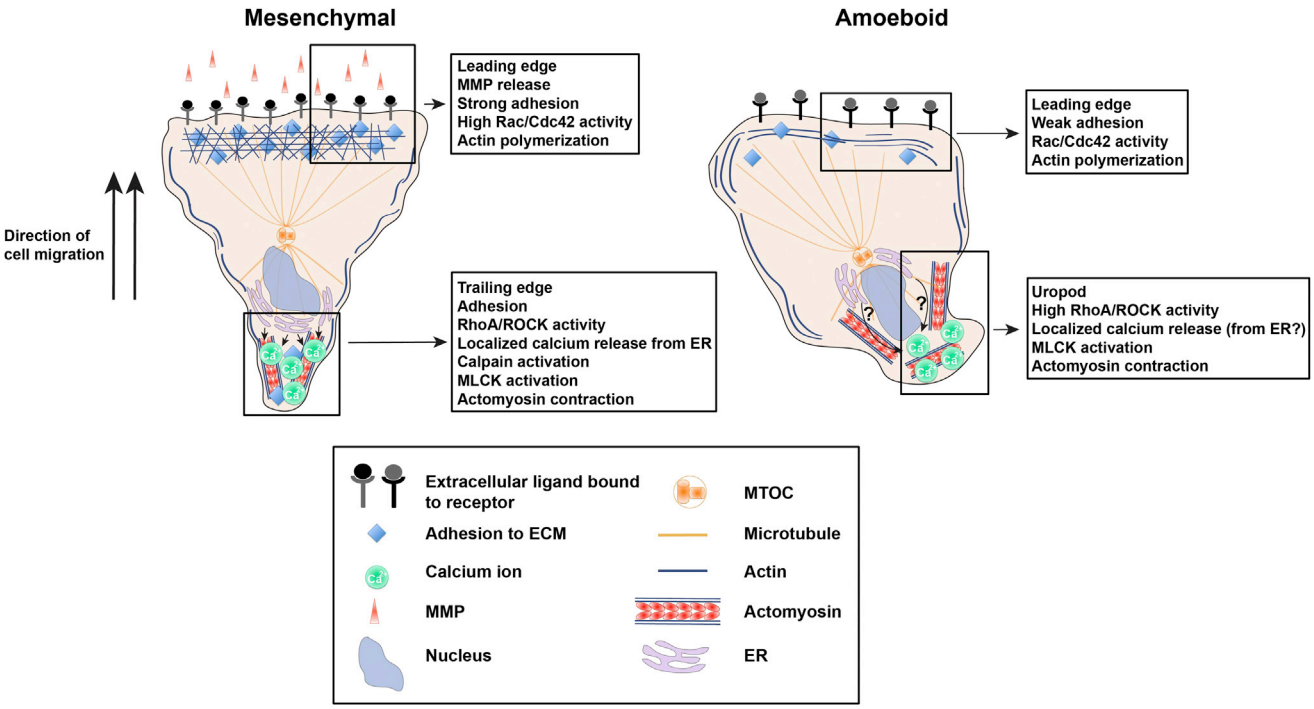

Figure 1. Mechanisms of Mesenchymal and Amoeboid Cell Migration

Both mesenchymal and amoeboid cell migration depend on formation of a leading and trailing edge. Mesenchymal cell migration relies on strong adhesion and actin polymerization that result from Rac/Cdc42 activation, which generates protrusions and drives motility forward at the cell's leading edge. In comparison, amoeboid cell migration is characterized by weak adhesions, high levels of Rho/ROCK activity, and actomyosin contraction at the trailing edge (uropod). Both modes of migration rely on localized calcium flux at the cell rear. Witze et al. (2013) showed that ER concentrates calcium at the trailing edge of cells during mesenchymal migration. How calcium is regulated at the uropod during amoeboid motility is still uncertain.

ER/Ca^{2+}-regulated targets would be different in these types of cells and may involve effects on actomyosin contraction rather than calpain-mediated proteolytic mechanisms. Alternatively, Ca^{2+} has been implicated in the recycling and trafficking of integrins at the uropod of migrating neutrophils. The presence of the ER within the WRAMP structure at the trailing edge would position key components in close proximity for this role. There is evidence to suggest that posterior localization of the ER may be highly conserved: components of the ER were found to localize to the uropod in the parasite *Entamoeba histolytica* after cell-surface receptors are activated with Concanavalin A (Girard-Misguich et al., 2008). In contrast to the WRAMP structure, the leukocyte uropod does not require microtubules because nocodazole can induce uropod formation and rapid neutrophil motility. Taken together, these findings provide further evidence to suggest that the uropod in rapidly motile cells may be fundamentally different from the cell posterior of mesenchymal cells (Sánchez-Madrid and Serrador, 2009).

In summary, Witze et al. (2013) describe a model in which an extracellular ligand, Wnt5a, connects polarization of the ER at the trailing edge and Ca^{2+} localization during directed cell migration (Figure 1). This model offers insight into how cell polarity allows for efficient motility by concentrating Ca^{2+} in localized regions within the cell's rear. This advance addresses a fundamental question: How does Ca^{2+} or a protease localize its function in a highly dynamic process such as cell motility? The segregation of distinct signaling domains is key—and the ER or other dynamic organelles are attractive candidates to mediate this kind of regulation. The extent to which ER localization precedes Ca^{2+} release at uropods of highly motile amoeboid cells is unknown and will be an important area for future investigation (Figure 1). There is recent evidence to suggest that membrane tension plays a key role in polarity regulation in rapidly motile cells such as neutrophils (Houk et al., 2012), raising questions about whether these types of mechanisms are also connected to ER-Ca^{2+}-dependent regulation.

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